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ON THE PHAGOCYTABILITY OF PNEUMOCOCCI IN THE SPUTUM IN PNEUMONIA.*

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PARK and Williams used the mass culture method¹ of inoculation into rabbits and white mice in order to determine the virulence of pneumococci isolated from sputum. The organisms so obtained from 87 per cent of pneumonic patients and from 69 per cent of healthy individuals were virulent when 4 c.c. were inoculated. When only 1 c.c. was inoculated 51 per cent of pneumonic patients and 31 per cent of healthy individuals showed virulent pneumococci. Buerger² obtained somewhat different results by injecting 24-hour pure cultures of pneumococci obtained from sputum into white mice. He found 79 per cent of virulent organisms in normal cases and 77 per cent in pneumonia cases. The results of these two independent researches show that the difference between the normal and pneumonic sputum in content of virulent pneumococci is at most only slight. Of further interest because showing a high percentage of virulent pneumococci in normal sputum is the work of Frost, Divine, and Reineking³ who found that 90 per cent of rabbits died within 24 hours after the injection of 2 c.c. of saliva of healthy persons intraperitoneally in the spring months; 73 per cent in the winter; and 42 per cent in the autumn. Only those animals were considered which showed a pneumococcus septicemia.

The object of the present work was to study further the virulence of pneumococci in pneumonic sputum, using susceptibility to phagocytosis as the criterion. Rosenow⁴ has shown that highly virulent pneumococci do not absorb opsonin in normal serum and resist

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¹"The mass culture method consists in inoculating a mass of sputum to be tested into serum-broth (previously tested for ability to give abundant growth of pneumococci), placing at 36° C. for 24 hours, and inoculating a certain amount of the resulting culture subcutaneously into the animal chosen. The culture isolated from the heart's blood of the animal at autopsy is then tested for virulence in the same species of animal."—Park and Williams, *Jour. Exper. Med.*, 1905, 7, p. 403.

² *Jour. Exper. Med.*, 1905, 7, p. 497.

³ *Jour. Infect. Dis.*, 1905, Supplement No. 1, p. 298.

⁴ *Jour. Infect. Dis.*, 1906, 3, p. 683.

phagocytosis; hence this method may be used for determining the virulence of single strains of organisms.

The series studied include 26 cases of lobar pneumonia, 16 before crisis, 4 on day of crisis, 5 after crisis, and 1 during lysis. They were chosen miscellaneously during February and March, 1907, from cases in hospitals. The duration of the disease varied from the second day after the chill to the fifth day after the crisis. In addition to these, two cases of bronchitis were studied and also two cases in which cultures were obtained post mortem from the tracheal and bronchial mucous membranes of individuals who died with lobar pneumonia.

In each case, except the two studied after death, the sputum was received in a sterile petri dish, washed in sterile broth, and immediately plated out in blood-agar; as soon as colonies appeared (always within 24 hours) subcultures upon blood-agar slants were made from six small green nonhemolyzing colonies.¹ On the following day opsonic experiments were made in the usual manner with normal human serum, human leucocytes, and a suspension of the bacteria in physiological salt solution. The mixtures were incubated for 30 minutes.

As a check on the identity of the organism each subculture was transferred to a tube of Hiss's inulin serum-water as modified by Ruediger.² This was particularly useful as a means of differentiating the pneumococcus from *Strept. viridans* and *Strept. mucosus*, two organisms described by Schottmüller which occur in the mouth and which also form slightly greenish colonies on blood-agar plates. If fermentation with acid production occurred in six days the organisms were considered to be pneumococci, and those organisms which failed to ferment inulin were left out of the final results.

In all 127 strains of organisms from pneumonic sputum and fulfilling these requirements were studied. No strain was allowed to grow

¹ See Schottmüller, *Munch. med. Wchnschr.*, 1903, 50, p. 849; Rosenow, *Jour. Infect. Dis.*, 1904, 1, p. 308.

² *Jour. Infect. Dis.*, 1906, 3, p. 755: "Dissolve 5 gr. of NaCl, 20 gr. of peptone (Witte), and 20 gr. of pure inulin in 1,000 c.c. of distilled water. Add 20 c.c. of a 5 per cent solution of pure litmus and tube, putting 2 c.c. of the mixture into each tube, and sterilize in the autoclav. After sterilization add (with a sterile pipette) 2 c.c. of sterile heated ascites fluid, or preferably heated beef serum, to each tube, and incubate for 24 hours before using. Great care must be observed not to use ascites fluid which contains fermentable carbohydrates. Each lot must be tested with organisms that are known to have great fermentative powers and if acid is produced it must not be used."

on artificial media for more than 72 hours before examination for fear of loss of virulence, although this danger is slight within a reasonable period, according to Rosenow¹ who in one case found a high degree of virulence preserved in growths upon blood agar after 163 days. Most of the strains were examined within 48 hours after collecting the sputum. Approximately uniform suspensions were used.

Of the total number of strains studied 119 were found to be easily susceptible to phagocytosis and only eight strains resisted phagocytosis or were taken up in very small numbers. In other words 93.7 per cent of organisms could be considered as avirulent, or only slightly virulent; and only 6.3 per cent of virulent organisms were found. The virulent organisms were obtained three hours before crisis, once during crisis and once four days after crisis.

In two cases of pneumonia several strains of pneumococci obtained from swab cultures of the tonsils were examined, cultures being taken at the same time from the sputum. No appreciable difference between the susceptibility to phagocytosis could be discovered between the organisms isolated from the tonsils and those from the sputum. Four strains were examined from two cases of acute bronchitis, and all were found to be easily susceptible to phagocytosis.

As a control of the reliability of estimating the virulence of pneumococci isolated from sputum by the susceptibility to phagocytosis two rabbits were injected intraperitoneally with 1 c.c. of a dense salt solution suspension of organisms from two different cases of pneumonia respectively. Both strains of organisms used were easily susceptible to phagocytosis and when injected into the animals had grown 48 hours upon blood-agar slants. One rabbit was unaffected except for a loss of 300 grams in weight. The other died after 28 days, and the pneumococcus was recovered in pure culture from the heart's blood. Therefore the virulence of the organisms must be assumed to have been of rather low grade.

Ten strains of pneumococci obtained post mortem from the tracheal and bronchial mucous membranes 12 hours after the death of a man from lobar pneumonia were uniformly readily susceptible to phagocytosis. Five cultures from the parenchyma of the lung were examined of which two were readily taken up by the leucocytes and

¹ *Jour. Infect. Dis.*, 1904, 1, p. 280.

three resisted phagocytosis. Five strains of pneumococci isolated from the pulmonary vein in the same case were all insusceptible to phagocytosis. Five strains isolated post mortem in another case from the tracheal and bronchial mucous membrane were all readily ingested by the leucocytes.

With a view to determining the possibility of increasing by symbiosis the virulence of organisms regarded as non-virulent two strains which were readily ingested by the leucocytes were grown together for 48 hours on blood-agar. At the expiration of this time they were still easily susceptible to phagocytosis. A subculture of one of these strains and of one obtained from the pulmonary vein post mortem were likewise grown together for 48 hours. Tests made with a suspension of the combined growth showed a complete resistance to phagocytosis. This result is in keeping with the finding of Rosenow¹ that an extract in salt solution of virulent pneumococci is capable of restoring a non-virulent strain to virulence and of rendering them insusceptible to phagocytosis. For it is possible that in this experiment the non-virulent organisms were made virulent by coming into contact with "virulin" from the strain which resisted phagocytosis. Of course other possibilities suggest themselves.

Assuming in the light of Rosenow's work that insusceptibility of pneumococci to phagocytosis may be considered as a criterion of virulence we see that by far the majority of pneumococci in the sputum of at least those cases of lobar pneumonia which I studied were non-virulent according to this standard. It is evident that no relation exists between the duration of pneumonia and the virulence, thus determined, of the organisms in the sputum. For as shown here, of the few strains that may be regarded as virulent some were obtained before crisis, one during crisis, and one as late as four days after crisis. These results differ radically from those of Park and Williams and the other authors I have cited, and this difference is no doubt owing to the difference in method.

The presence of so large a percentage of non-virulent pneumococci in pneumonic sputum, i. e., non-virulent because susceptible to phagocytosis, seems to me to be rather surprising because in practically all cases of lobar pneumonia there is a pneumococcemia of virulent

¹ *Jour. Infect. Dis.*, 1907, 4, p. 285.

organisms, i. e., those insusceptible to the action of normal opsonin (Rosenow). Naturally one would expect a considerable number of such organisms in the sputum of pneumonic patients. The strains isolated from the pulmonary vein in a case of pneumonia were uniformly insusceptible to phagocytosis while strains from the tracheal and bronchial membranes of the same case were readily phagocyttable. It lies near at hand to assume that perhaps only virulent organisms gain access to the blood stream. Another possible explanation is that organisms from the blood stream are excreted through the respiratory mucous membranes after being so changed that they lose the virulent property by virtue of which they resist the action of opsonin. In this connection the observations of Buerger and Ryttenberg¹ are of great interest. These observers found that pneumococci in the blood of a patient with a general infection with this organism may differ in cultural characteristics from those obtained from the metastatic foci, showing that the medium has a pronounced effect upon the biological properties of the pneumococcus even when it is growing in the living body. A third possibility also suggests itself, namely that mucus and saliva may possess properties that render virulent pneumococci phagocyttable if sufficient time is given. In the latter case it could be assumed that the virulent organisms encountered in the sputum are the last ones to come into contact with the mucus and saliva. With this last idea in view the following experiment was made: A strain of virulent pneumococci obtained by blood culture from a case of lobar pneumonia was suspended in salt solution to which an equal portion of filtered normal saliva was added. Blood-agar plates were made $\frac{1}{2}$ hour, $3\frac{1}{2}$ hours, and $17\frac{1}{2}$ hours later. The suspensions were kept at room temperature. After plating the cultures in each instance they were incubated at 37°C . for 24 hours. The plates made from the mixture of organisms and saliva after $\frac{1}{2}$ hour showed 70 small green colonies; those made at the end of $3\frac{1}{2}$ hours showed 20 colonies and in those plates made at the expiration of $17\frac{1}{2}$ hours no colonies were present. In other words in a single experiment no pneumococci developed after being in contact with normal saliva for $17\frac{1}{2}$ hours. The result suggests the presence in saliva of a pneumococcidal substance. The experiments I have been able to make are, however, too few to justify final conclusions.

¹ *Jour. Infect. Dis.*, 1907, 4, p. 609.

SUMMARY.

In lobar pneumonia pneumococci isolated from the blood stream are insusceptible to the action of normal opsonin, while the great majority of those obtained from pneumonic sputum are readily phagocytatable under the influence of normal serum.

In 127 strains of pneumococci isolated from sputum of 26 cases of lobar pneumonia 93.7 per cent were phagocytatable hence non-virulent, and 6.3 per cent were non-phagocytatable hence virulent. The duration of the disease seemed to make no appreciable difference in the virulence of the organisms isolated. Cultures from the tonsils, the trachea, and the bronchi, even in fatal cases, showed no increase in the percentage of virulent strains as compared with cultures obtained from sputum.

This fundamental difference between the pneumococci in the blood and in the sputum and respiratory tract in pneumonia merits further investigation.